

Prion-like propagation of cytosolic protein aggregates

Insights from cell culture models

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Abbreviations: Htt, huntingtin; PrP, prion protein; polyQ, polyglutamine; MTBR, microtubule binding region of Tau; CFP, cyan fluorescent protein; GFP, green fluorescent protein; chFP, cherry fluorescent protein; HA, hemagglutinin antibody epitope

Amyloid formation is a hallmark of several systemic and neurodegenerative diseases. Extracellular amyloid deposits or intracellular inclusions arise from the conformational transition of normally soluble proteins into highly ordered fibrillar aggregates. Amyloid fibrils are formed by nucleated polymerization, a process also shared by prions, proteinaceous infectious agents identified in mammals and fungi. Unlike so called non-infectious amyloids, the aggregation phenotype of prion proteins can be efficiently transmitted between cells and organisms. Recent discoveries in vivo now implicate that even disease-associated intracellular protein aggregates consisting of α -synuclein or Tau have the capacity to seed aggregation of homotypic native proteins and might propagate their amyloid states in a prion-like manner. Studies in tissue culture demonstrate that aggregation of diverse intracellular amyloidogenic proteins can be induced by exogenous fibrillar seeds. Still, a prerequisite for prion-like propagation is the fragmentation of proteinaceous aggregates into smaller seeds that can be transmitted to daughter cells. So far efficient propagation of the aggregation phenotype in the absence of exogenous seeds was only observed for a yeast prion domain expressed in tissue culture. Intrinsic properties of amyloidogenic protein aggregates and a suitable host environment likely determine if a protein polymer can propagate in a prion-like manner in the mammalian cytosol.

Infectious and Non-Infectious Amyloids

Amyloidoses are degenerative diseases that are associated with fibrillar aggregates of diverse host-encoded proteins. More than 30 human diseases are associated with amyloid deposition, highly ordered fibrillar aggregates with cross β -sheet structure that are formed by proteins with little sequence similarity.¹ Amyloid diseases comprise both systemic and neurologic diseases such as

Alzheimer disease, Parkinson disease and Chorea Huntington.² The fundamental event underlying amyloidoses is the aggregation of aberrantly folded proteins or peptides into amyloid polymers. According to the nucleated polymerization model, seeds composed of abnormally folded proteins template the conformational transition of soluble cognate proteins (Fig. 1A).³ The mechanism of nucleated polymerization is also shared by prions, proteinaceous infectious proteins⁴ (Fig. 1B) so far identified in mammals and lower eukaryotes. Amyloid diseases and prion diseases can be sporadic or heritable. The infectious nature ultimately separates prion diseases from other amyloidoses. Prion diseases in mammals are fatal neurodegenerative disorders that affect both humans and animals.⁵⁻⁷ Similar to other amyloidoses, human prion diseases can arise spontaneously with an incidence of approx. 1:10⁶ or are inherited due to germline mutations in the prion protein coding sequence.⁸ Importantly, prions are also infectious and transmit experimentally or naturally within or even between species. It is now widely accepted that an abnormal isoform of the host-encoded prion protein PrP^{Sc} constitutes the causative agent of prion diseases. Abnormal PrP (PrP^{Sc}) acts as a template that recruits and converts normal prion protein (PrP^C) into a structured PrP^{Sc} polymer. Strong support for the protein-only hypothesis comes from several recent studies on in vitro generated prion infectivity.⁹⁻¹¹ PrP^C is a glycosylated protein attached to the outer leaflet of the cell membrane by a glycosyl-phosphatidylinositol-anchor. Conversion of PrP^C to PrP^{Sc} likely occurs on the cell surface or within early endosomal compartments.¹²⁻¹⁴

Remarkably, self-perpetuating prion states have also been demonstrated for a growing class of diverse proteins in lower eukaryotes.¹⁵ Rather than causing disease, fungal prions constitute epigenetic elements of inheritance that can “encode” and transmit conformational information. In most cases, a conformational switch of the normally soluble and functional prion protein inactivates the protein and leads to a change in phenotype. The induction of the prion phenotype can be favorable under some growth conditions.¹⁶ Thus, fungal prions “act as genes” that, although their information content is lower than nucleic

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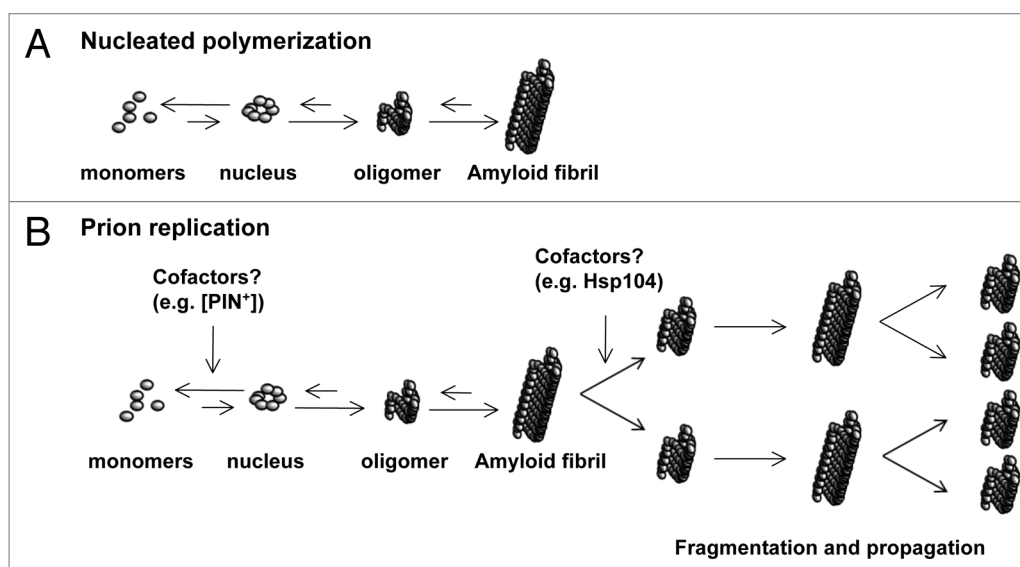


Figure 1. Schematic representation of amyloid and prion formation. (A) Assembly of amyloid fibrils follows a nucleated polymerization reaction with a lag phase required to form an aggregation nucleus followed by an elongation phase in which monomers are rapidly recruited into growing aggregates. Oligomers are common folding intermediates of amyloid fibrils. Amyloid fibrils have a cross- β structure, bind to fluorophore thioflavine T and exhibit Congo Red birefringence. (B) Efficient prion propagation follows the nucleated polymerization process. Spontaneous induction of prion protein nuclei is a rare event. In yeast, the prion phenotype [PIN⁺] of the host protein Rnq1 strongly enhances the de novo appearance of other prions (e.g., the prion conformation of Sup35, [PSI⁺]), likely by cross-seeding. Induction mechanisms in spontaneous human prion diseases are unknown. The disaggregase Hsp104 is essential for partitioning large prion polymers into smaller propagons that are transmitted during cell division or mating in yeast. Essential co-factors for mammalian prion propagation remain elusive.

acid, may allow rapid adaption to environmental changes.^{15,17} However, the fact that the [PSI⁺] and [URE3] prion phenotypes are not prevalent in wild strain isolates suggests that they might not be beneficial under all circumstances.¹⁸ Reversion between the non-prion state and the prion state can occur sporadically at very low rate.¹⁹ Overexpression of soluble homotypic protein increases rates with which the prion phenotypes arise de novo, likely due to misfolding of overproduced protein.^{15,20} Spontaneous induction is strongly enhanced by the prion state of the yeast protein Rnq1 and prion aggregates thereof appear to cross-seed aggregation of several other prion proteins.²¹ Naturally, yeast prions transmit to daughter cells during cell division and the prion phenotype is heritably propagated. New rounds of prion replication are initiated by segregation of prion polymers into smaller seeds, a process that crucially involves the disaggregase Hsp104 and other members of the chaperone machinery.²² Yeast prions are considered infectious because the prion phenotype can be experimentally transmitted to naïve yeast cells by a process called cytoduction.²³

Classical Amyloidoses Share some Features with Prions

The discrimination between non-infectious aggregation-prone proteins and prions has been challenged by recent findings that both systemic and tissue-specific amyloidoses can be induced by seeding-like mechanisms that share similarities with prion propagation.²⁴⁻²⁶ Degeneration of interconnected brain regions has been reported for several amyloid-related neurodegenerative disorders, including progressive supranuclear palsy and progressive

tauopathy in Alzheimer disease. The observed network degeneration²⁷ implicates spreading of protein misfolding rather than cell autonomous intracellular protein aggregate formation. Indeed, tauopathy was shown to be transmittable experimentally by injecting transgenic mouse brain extracts that contain mutant P301S Tau fibrils into mice expressing wild-type human Tau.²⁸ Wild-type Tau aggregation was triggered at the site of injection and at more distant areas, strongly suggesting that protein-misfolding was capable of spreading within the brain.²⁸ Moreover, inclusions of aggregated α -synuclein, a major component of intracellular Lewy bodies associated with several neurodegenerative diseases, were found in neurons that were grafted into the brain of Parkinson disease patients, implicating transmission of α -synuclein misfolding from host to mesencephalic transplants.^{29,30} These findings provoke the question whether the concept of infectious protein conformers may also be applicable to a broader spectrum of amyloids.

Exogenous Amyloid Fibrils can Seed Aggregation of Cognate Proteins in the Cytosol

Clearly, the findings that Tau and α -synuclein aggregation spreads in connected brain areas raise the important question how intracellular protein aggregates can trigger aggregation of cognate soluble proteins in neighboring cells. Cytosolic aggregate formation of Tau, α -synuclein and Huntingtin can also be recapitulated in cell culture by overexpression of wild-type proteins, mutant proteins or disease-associated fragments thereof. Expression of these proteins frequently leads to the formation of

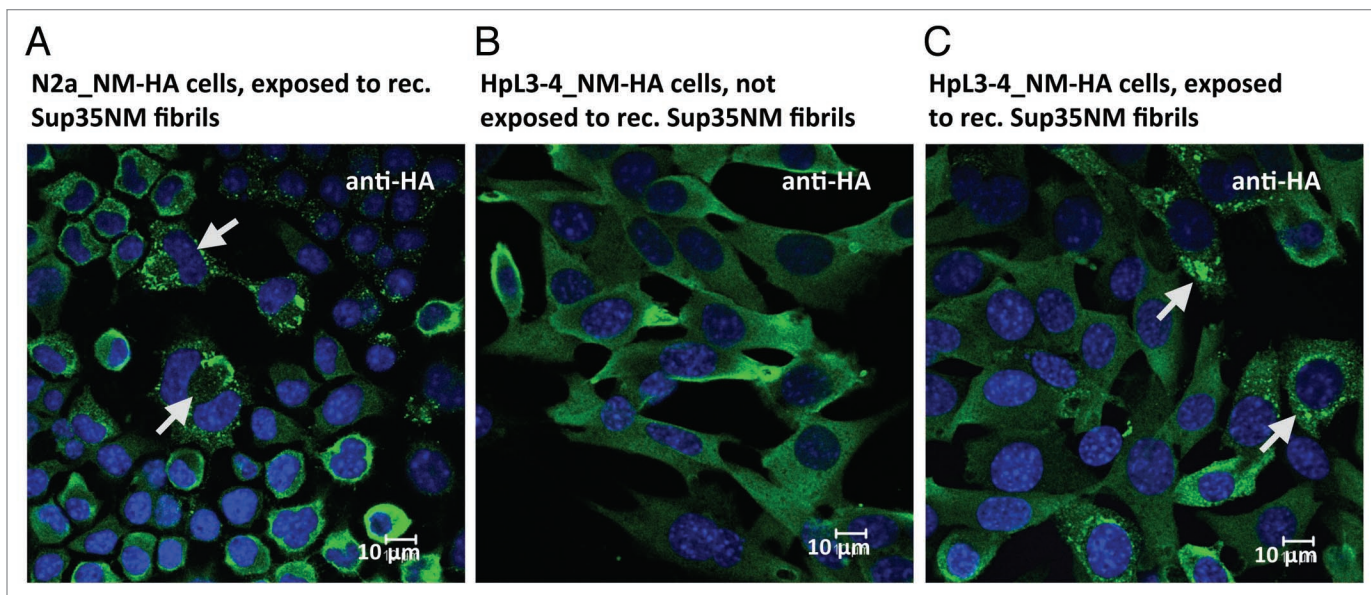


Figure 2. Propagation of NM-HA aggregates in mammalian cells. (A) N2a cells stably expressing NM-HA were treated with in vitro generated fibrils of recombinant Sup35NM and subsequent passages were analyzed by confocal microscopy. Once induced, NM-HA aggregates were efficiently transmitted to daughter cells during cell division. Arrows mark cells that undergo cell division. (B) NM-HA is soluble when stably expressed in HpL3-4 cells. (C) Induction and propagation of NM-HA aggregates in HpL3-4 cells. Two passages after treatment with recombinant Sup35NM fibrils, HpL3-4_NM-HA cells exhibit NM-HA aggregates (marked by arrows). Antibody, anti-HA. Nuclei were visualized using Hoechst dye.

so-called aggresomes, pericentriolar inclusions that arise when the ubiquitin-proteasome system that normally degrades misfolded proteins is impaired.³¹ Aggresome formation is an active process of the cell to sequester otherwise detrimental protein aggregates for storage or subsequent clearance by autophagosomes.³²

Recent data now provide evidence that exogenous proteinaceous aggregates can trigger cytosolic assembly of aggregation-prone proteins that are normally soluble. Amyloidogenic proteins in their monomeric or polymeric states have been shown to be internalized by cells.³³⁻⁴¹ Recombinant fibrils derived from polyglutamine peptides, the microtubule binding region of Tau (MTBR), A β and the prion domain of the *Saccharomyces cerevisiae* Sup35 were readily internalized by tissue culture cells and translocated to the cytosol.⁴²⁻⁴⁵ But how exactly do apparently high molecular protein aggregates penetrate the cell? PrP^{Sc} has been repeatedly shown to be taken up by various cell types in vitro^{46,47} and co-localization with endosomal markers or uptake inhibition at low temperatures implicates involvement of the endosomal pathway.^{48,49} Likewise, MTBR in its fibrillar state was internalized by fluid phase endocytosis.⁴⁵ The transmission of α -synuclein polymers was reduced considerably in different cell types expressing a dominant negative mutant of dynamin, suggesting that internalization was dependent on endocytic processes.^{39,41} By contrast, Ren et al. suggested direct penetration of the lipid bilayer as a mechanism for polyglutamine fibril translocation to the cytoplasm. Importantly, membrane permeabilization was also observed for some oligomers from α -synuclein, polyglutamine peptides and prion protein peptide 106–126 but not for fibrils.^{40,50,51} Internalization mechanisms may therefore differ depending on the size and structure of the polymer, the protein aggregate or the cell-type.

Exogenous oligomers or amyloid-like fibrils can also initiate aggregation of cytosolic homotypic proteins in tissue culture models. Early evidence that extracellular protein polymers can induce intracellular aggregation of homotypic proteins came from studies with different α -synuclein oligomers. Intriguingly, some but not all types of α -synuclein oligomers derived from recombinant protein were capable of inducing intracellular wild-type and mutant α -synuclein inclusions.⁴⁰ We have previously shown that the yeast Sup35NM prion domain tagged with the antibody epitope of hemagglutinin (NM-HA) remains soluble when expressed cytosolically in murine N2a neuroblastoma cells.⁵² Addition of exogenous recombinant fibrils but not monomeric Sup35NM to the cell culture medium efficiently triggered aggregation of the cognate soluble protein (Fig. 2A).⁴³ Induction of NM-HA aggregates was also observed in a PrP deficient hippocampal cell line stably expressing NM-HA, demonstrating that both Sup35NM fibril uptake and propagation of the NM-HA aggregate phenotype occurred in another cell line and were independent of the expression of the endogenous prion protein PrP (Fig. 2B and C). Seeding of intracellular aggregates by external amyloid fibrils was also recently shown for two other disease-related amyloidogenic proteins. Self-assembly into cytosolic or nucleoplasmic inclusions is drastically increased with proteins exhibiting polyglutamine (polyQ) tracts above a pathogenic threshold of approx. 37 repeats. Pathogenic polyQ proteins have been shown to recruit non-pathogenic soluble polyQ proteins into juxtanuclear inclusions when proteins are co-expressed in mammalian cells.^{53,54} Interestingly, exogenous recombinant polyQ (Q44) fibrils were taken up by the cell and sequestered ectopically expressed soluble cyan fluorescent protein tagged Htt exon 1 (CFP-HttQ25) into aggresomes⁴⁴ (Fig.

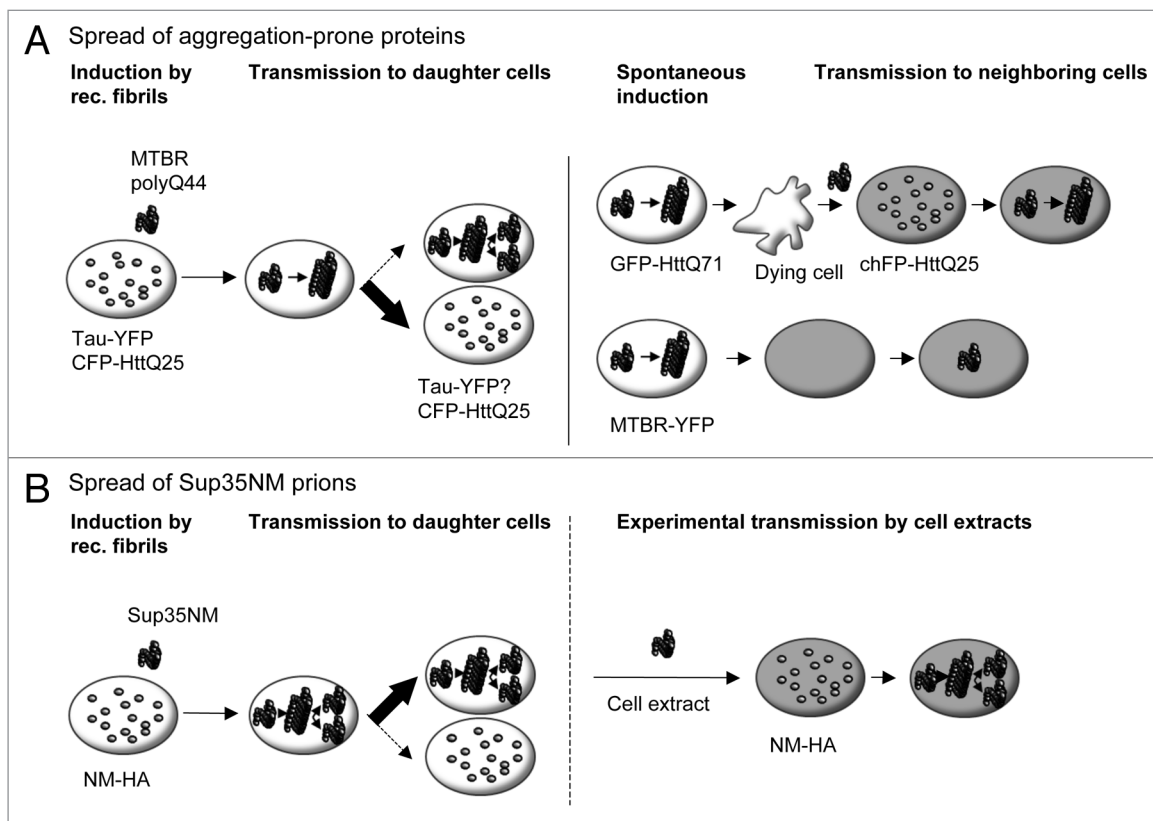


Figure 3. Comparison of aggregation and dissemination propensities of cytosolic aggregation-prone proteins and Sup35NM prions in cell culture. (A) Induction of homotypic protein aggregates and transfer of aggregates to recipient cells. Left, yellow-fluorescent protein-tagged wild-type Tau (Tau-YFP) and cyan-fluorescent protein tagged Huntingtin fragment HttQ25 (CFP-HttQ25) exhibit low spontaneous aggregation propensities.^{44,45} Uptake of fibrillar aggregates of recombinant polyglutamine peptides (polyQ44) or the microtubule binding region of Tau (MTBR) triggers co-aggregation with soluble cognate proteins. Induced CFP-HttQ25 aggregates display low mitotic stability. Maintenance of wild-type Tau aggregates has not been assessed. Right: GFP-HttQ71 aggregates formed spontaneously upon transient expression and released by dying cells can be taken up by co-cultured recipient cells where they trigger aggregation of soluble cherry fluorescent protein-tagged HttQ25 (chFP-HttQ25). Recipient cells are marked in grey. Similarly, YFP-tagged MTBR aggregates (MTBR-YFP) spontaneously formed upon transient expression in cell culture are transferred to recipient cells. (B) Sup35NM prion propagation. Spontaneous formation of NM-HA aggregates is a very rare event.⁵² Aggregation of NM-HA can be initiated by recombinant Sup35NM fibrils.⁴³ Once induced, the prion phenotype is maintained during cell division probably due to a very effective mechanism of aggregate fractionation and dissemination. Cell-derived Sup35NM prions are experimentally transmissible to mammalian cells expressing soluble NM-HA and to a lower extent to yeast. Mechanisms of cell-to-cell transmission in cell culture are currently under investigation.

3A, left). Seeding of intracellular protein aggregates by external amyloid fibrils was also demonstrated in a cell culture model for Tau aggregation.⁴⁵ Transiently expressed wild-type Tau exhibits a low tendency to spontaneously self-assemble into aggregates, while the microtubule binding region MTBR readily forms inclusions in cell culture. However, exposure of cells to recombinant MTBR fibrils induced aggregation of endogenous wild-type Tau (Fig. 3A, left).

Importantly, spontaneously formed MTBR aggregates were also naturally transferred between cells, suggesting that transmission of protein aggregates could also initiate aggregation of Tau in neighboring cells (Fig. 3A, right). Natural transmission of Htt polyglutamine polymers in cell culture was rather inefficient but could be drastically increased by disruption of co-cultured donor cells⁴⁴ (Fig. 3A, right). Transmission of donor-cell derived α -synuclein and subsequent inclusion body formation was also observed in co-culturing experiments with cells overexpressing α -synuclein and control cells, suggesting that aggregates were

transmitted between cells.⁴¹ If α -synuclein transmission in cell culture can also initiate aggregation of soluble homotypic protein, it has not been elucidated so far. Cell-to-cell transmission of NM-HA aggregates in cell culture is currently under investigation. However, extracts from cells displaying NM-HA aggregates can induce NM-HA aggregation in recipient mammalian cells, providing experimental evidence that the cell-derived aggregates are taken up by cells (Fig. 3B, right). But how are protein aggregates spread between cells? Exocytosis of α -synuclein in its monomeric and aggregated state was recently demonstrated.⁵⁵ Efficient spread of mammalian prions at least in tissue culture is achieved by direct cell-to-cell contact, for example by nanotubes,^{56,57} or by release of prion infectivity into the medium, likely by exosomes.⁵⁸⁻⁶¹ Similar mechanisms might also account for the transfer of other protein aggregates within cell populations.

Mitotic Stability of Protein Aggregates is Indispensable for Stable Prion Propagation in Tissue

Culture

Induction of cytosolic protein aggregation by external amyloid seeds and transfer of proteinaceous aggregates between cells are characteristic features of the prion life cycle. However, mitotic stability of protein aggregates is ultimately required for stable prion maintenance in tissue culture and yeast. Once infected, cells can produce infectious prions over many cell generations.^{19,62} Remarkably, a transient exposure of N2a cells expressing NM-HA to recombinant Sup35NM fibrils was sufficient to initiate self-seeded propagation of NM-HA aggregates that was maintained over continuous cell divisions (Fig. 3B, left). Intriguingly, NM-HA aggregates induced by recombinant fibrils varied from frequent small punctate aggregates to few long, unbranched polymers. Single cell cloning revealed that different types of aggregates were faithfully propagated by progeny cells without any obvious loss of the prion phenotype. The fact that extracts from N2a cells containing NM-HA aggregates were also capable of inducing the prion phenotype in yeast (albeit at a low rate) argues that aggregates formed in mammalian cells share features with Sup35 yeast prions. In conclusion, NM-HA aggregates induced in mammalian cells fulfill several criteria for prions: (1) spontaneous prion induction is an extremely rare event, (2) the prion phenotype can be induced by external seeds, (3) prions are stably maintained during cell division and (4) prions are transmissible to naïve cells where they initiate continuous aggregation of normally soluble homotypic proteins.

If amyloidogenic proteins such as α -synuclein or Tau have the capacity to trigger a self-perpetuating aggregation mechanism that progresses independent of exogenous seeds remains to be determined. The high penetrance of the NM-HA prion phenotype is in strong contrast to the low persistence of CFP-HttQ25 aggregates induced by recombinant polyglutamine fibrils (approx., 3–8% of cells with persistent inclusions in induced cells versus 0–4% of inclusions in non-induced control cells).⁴⁴ Asymmetrical distribution of polyglutamine inclusions between daughter cells was also observed in tissue culture and a *Drosophila* model of Huntington disease,⁶³ suggesting that except for a small fraction, induced CFP-HttQ25 aggregates might have been diluted out in subsequent cell passages.

The inefficient propagation of induced polyglutamine aggregates and the formation of polyglutamine aggresomes led to the proposal that mammalian cells might have evolved efficient mechanisms to prevent prion-like aggregate propagation in the cytosol.⁴⁴ Our studies on the aggregation behavior of the yeast Sup35NM prion domain clearly demonstrate that at least some cytosolic proteinaceous aggregates can escape the quality control system of the cell and can be stably maintained upon continuous passage once initiated. If NM-HA aggregates display any characteristics of aggresomes needs further investigation, but clearly single juxtanuclear NM-HA structures were hardly ever detected. Remarkably, transient overexpression of huntingtin exon I with

an extended polyglutamine stretch (HD72Q-GFP) in cells stably expressing NM-HA also led to co-aggregation with NM-HA.⁶⁴ However, aggregation of NM-HA depended on the continuous presence of HD72Q-GFP and was lost once the polyglutamine polypeptide was no longer expressed.⁶⁴ A high content of glutamine might thus initiate self-assembly of aggregation-prone proteins with similar aggregation-prone stretches, but might be insufficient to allow stable propagation of the aggregated state in a prion-like manner. The fact that NM-HA aggregates induced by recombinant Sup35NM fibrils were not diluted during cell division but efficiently transmitted to daughter cells in subsequent passages (Fig. 2) implicates that aggregates display an intrinsic fragility and/or polymers are efficiently segregated into smaller seeds that can be transmitted during cell division, as has been shown for fungal prions. How this is achieved and if the cellular chaperone machinery is involved remains to be elucidated. Fragmentation of yeast prions crucially depends on Hsp104,^{22,65–68} a chaperone with disaggregating activity with no known orthologs in the mammalian cytosol. Several other chaperones with homologs in mammals are involved in yeast prion propagation.^{69,70} Although it was shown that the aggregation domain of Sup35 can to some extent be replaced by a polyQ tract,⁷¹ amino acid composition differences in polyQ stretches and the prion domain of Sup35NM account for the difference in initiation of aggregation and mitotic stability of the respective protein aggregates in yeast.^{71–73} A recent systematic approach revealed a strong bias towards asparagines versus glutamines in identified prion domains in yeast.⁷⁴ Which sequences allow efficient prion replication in the mammalian cytosol remains to be shown. However, the interplay between intrinsic properties of amyloidogenic proteins and cellular factors in the host cell environment likely accounts for the efficient propagation of the prion state in the mammalian cytosol. Importantly, the prion-like replication of NM-HA aggregates in N2a cells argues that transmission of structural information might also play a physiological role for certain so-far unidentified mammalian proteins, as has been demonstrated for proteins in lower eukaryotes.^{75,76} It will be one of the major challenges of the future to identify and experimentally validate mammalian proteins with prion-forming capacities and to dissect the cellular machinery that enables their replication as prions.

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References

- Dobson CM. Protein aggregation and its consequences for human disease. *Protein Pept Lett* 2006; 13:219-27.
- Chiti F, Dobson CM. Protein misfolding, functional amyloid, and human disease. *Annu Rev Biochem* 2006; 75:333-66.
- Harper JD, Lansbury PT Jr. Models of amyloid seeding in Alzheimer's disease and scrapie: mechanistic truths and physiological consequences of the time-dependent solubility of amyloid proteins. *Annu Rev Biochem* 1997; 66:385-407.
- Prusiner SB. Novel proteinaceous infectious particles cause scrapie. *Science* 1982; 216:136-44.
- Aguzzi A, Polymenidou M. Mammalian prion biology: one century of evolving concepts. *Cell* 2004; 116:313-27.
- Prusiner SB. Prions. *Proc Natl Acad Sci USA* 1998; 95:13363-83.
- Weissmann C. The state of the prion. *Nat Rev Microbiol* 2004; 2:861-71.
- Collinge J. Prion diseases of humans and animals: their causes and molecular basis. *Annu Rev Neurosci* 2001; 24:519-50.
- Castilla J, Saa P, Hetz C, Soto C. In vitro generation of infectious scrapie prions. *Cell* 2005; 121:195-206.
- Legname G, Baskakov IV, Nguyen HO, Riesner D, Cohen FE, Dearmond SJ, Prusiner SB. Synthetic mammalian prions. *Science* 2004; 305:673-6.
- Deleault NR, Harris BT, Rees JR, Supattapone S. Formation of native prions from minimal components in vitro. *Proc Natl Acad Sci USA* 2007; 104:9741-6.
- Caughey B. Scrapie associated PrP accumulation and its prevention: insights from cell culture. *Br Med Bull* 1993; 49:860-72.
- Krammer C, Vorberg I, Schatzl HM, Gilch S. Therapy in prion diseases: from molecular and cellular biology to therapeutic targets. *Infect Disord Drug Targets* 2009; 9:3-14.
- Marijanovic Z, Caputo A, Campana V, Zurzolo C. Identification of an intracellular site of prion conversion. *PLoS Pathog* 2009; 5:1000426.
- Wickner RB. [URE3] as an altered URE2 protein: evidence for a prion analog in *Saccharomyces cerevisiae*. *Science* 1994; 264:566-9.
- True HL, Lindquist SL. A yeast prion provides a mechanism for genetic variation and phenotypic diversity. *Nature* 2000; 407:477-83.
- Shorter J, Lindquist S. Prions as adaptive conduits of memory and inheritance. *Nat Rev Genet* 2005; 6:435-50.
- Nakayashiki T, Kurtzman CP, Edsles HK, Wickner RB. Yeast prions [URE3] and [PSI⁺] are diseases. *Proc Natl Acad Sci USA* 2005; 102:10575-80.
- Cox BS, Byrne LJ, Tuite MF. Prion stability. *Prion* 2007; 1:170-8.
- Chernoff YO, Derkach IL, Inge-Vechtomov SG. Multicopy SUP35 gene induces de-novo appearance of psi-like factors in the yeast *Saccharomyces cerevisiae*. *Curr Genet* 1993; 24:268-70.
- Derkach IL, Bradley ME, Hong JY, Liebman SW. Prions affect the appearance of other prions: the story of [PIN(+)]. *Cell* 2001; 106:171-82.
- Chernoff YO, Lindquist SL, Ono B, Inge-Vechtomov SG, Liebman SW. Role of the chaperone protein Hsp104 in propagation of the yeast prion-like factor [psi⁺]. *Science* 1995; 268:880-4.
- Wickner RB, Edsles HK, Shewmaker F. How to find a prion: [URE3], [PSI⁺] and [beta]. *Methods* 2006; 39:3-8.
- Lundmark K, Westermark GT, Nystrom S, Murphy CL, Solomon A, Westermark P. Transmissibility of systemic amyloidosis by a prion-like mechanism. *Proc Natl Acad Sci USA* 2002; 99:6979-84.
- Kane MD, Lipinski WJ, Callahan MJ, Bian F, Durham RA, Schwarz RD, et al. Evidence for seeding of beta-amyloid by intracerebral infusion of Alzheimer brain extracts in beta-amyloid precursor protein-transgenic mice. *J Neurosci* 2000; 20:3606-11.
- Meyer-Luehmann M, Coomaraswamy J, Bolmont T, Kaeser S, Schaefer C, Kilger E, et al. Exogenous induction of cerebral beta-amyloidogenesis is governed by agent and host. *Science* 2006; 313:1781-4.
- Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging* 2003; 24:197-211.
- Clavaguera F, Bolmont T, Crowther RA, Abramowski D, Frank S, Probst A, et al. Transmission and spreading of tauopathy in transgenic mouse brain. *Nat Cell Biol* 2009; 11:909-13.
- Li JY, Englund E, Holton JL, Soulet D, Hagell P, Lees AJ, et al. Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. *Nat Med* 2008; 14:501-3.
- Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow CW. Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nat Med* 2008; 14:504-6.
- Kopito RR. Aggresomes, inclusion bodies and protein aggregation. *Trends Cell Biol* 2000; 10:524-30.
- Iwata A, Christianson JC, Bucci M, Ellerby LM, Nukina N, Forno LS, Kopito RR. Increased susceptibility of cytoplasmic over nuclear polyglutamine aggregates to autophagic degradation. *Proc Natl Acad Sci USA* 2005; 102:13135-40.
- Burdick D, Kosmoski J, Knauer MF, Glabe CG. Preferential adsorption, internalization and resistance to degradation of the major isoform of the Alzheimer's amyloid peptide, Abeta 1-42, in differentiated PC12 cells. *Brain Res* 1997; 746:275-84.
- Sung JY, Kim J, Paik SR, Park JH, Ahn YS, Chung KC. Induction of neuronal cell death by Rab5A-dependent endocytosis of alpha-synuclein. *J Biol Chem* 2001; 276:27441-8.
- Nagele RG, D'Andrea MR, Anderson WJ, Wang HY. Intracellular accumulation of beta-amyloid(1-42) in neurons is facilitated by the alpha7 nicotinic acetylcholine receptor in Alzheimer's disease. *Neuroscience* 2002; 110:199-211.
- Bucciantini M, Calloni G, Chiti F, Formigli L, Nosi D, Dobson CM, Stefani M. Prefibrillar amyloid protein aggregates share common features of cytotoxicity. *J Biol Chem* 2004; 279:31374-82.
- Ahn M, Kim S, Kang M, Ryu Y, Kim TD. Chaperone-like activities of alpha-synuclein: alpha-synuclein assists enzyme activities of esterases. *Biochem Biophys Res Commun* 2006; 346:1142-9.
- Saavedra L, Mohamed A, Ma V, Kar S, de Chaves EP. Internalization of beta-amyloid peptide by primary neurons in the absence of apolipoprotein E. *J Biol Chem* 2007; 282:35722-32.
- Lee HJ, Suk JE, Bae EJ, Lee SJ. Clearance and deposition of extracellular alpha-synuclein aggregates in microglia. *Biochem Biophys Res Commun* 2008; 372:423-8.
- Danzer KM, Haasen D, Karow AR, Moussaud S, Habbeck M, Giese A, et al. Different species of alpha-synuclein oligomers induce calcium influx and seeding. *J Neurosci* 2007; 27:9220-32.
- Desplats P, Lee HJ, Bae EJ, Patrick C, Rockenstein E, Crews L, et al. Inclusion formation and neuronal cell death through neuron-to-neuron transmission of [alpha]-synuclein. *Proc Natl Acad Sci USA* 2009.
- Yang W, Dunlap JR, Andrews RB, Wetzel R. Aggregated polyglutamine peptides delivered to nuclei are toxic to mammalian cells. *Hum Mol Genet* 2002; 11:2905-17.
- Krammer C, Kryndushkin D, Suhre MH, Krammer E, Hofmann A, Pfeifer A, et al. The yeast Sup35NM domain propagates as a prion in mammalian cells. *Proc Natl Acad Sci USA* 2009; 106:462-7.
- Ren PH, Lauckner JE, Kachirskaya I, Heuser JE, Melki R, Kopito RR. Cytoplasmic penetration and persistent infection of mammalian cells by polyglutamine aggregates. *Nat Cell Biol* 2009; 11:219-25.
- Frost B, Jacks RL, Diamond MI. Propagation of tau misfolding from the outside to the inside of a cell. *J Biol Chem* 2009; 284:12845-52.
- Hijazi N, Kariv-Inbal Z, Gasset M, Gabizon R. PrP^{Sc} incorporation to cells requires endogenous glycosaminoglycan expression. *J Biol Chem* 2005; 280:17057-61.
- Horonchik L, Tzaban S, Ben Zaken O, Yedidia Y, Rouvinski A, Papy-Garcia D, et al. Heparan sulfate is a cellular receptor for purified infectious prions. *J Biol Chem* 2005; 280:17062-7.
- Magalhaes AC, Baron GS, Lee KS, Steele-Mortimer O, Dourward D, Prado MA, Caughey B. Uptake and neuritic transport of scrapie prion protein coincident with infection of neuronal cells. *J Neurosci* 2005; 25:5207-16.
- Greil CS, Vorberg IM, Ward AE, Meade-White KD, Harris DA, Priola SA. Acute cellular uptake of abnormal prion protein is cell type and scrapie-strain independent. *Virology* 2008; 379:284-93.
- Volles MJ, Lee SJ, Rochet JC, Shitlerman MD, Ding TT, Kessler JC, Lansbury PT Jr. Vesicle permeabilization by protofibrillar alpha-synuclein: implications for the pathogenesis and treatment of Parkinson's disease. *Biochemistry* 2001; 40:7812-9.
- Kayed R, Sokolov Y, Edmonds B, McIntire TM, Milton SC, Hall JE, Glabe CG. Permeabilization of lipid bilayers is a common conformation-dependent activity of soluble amyloid oligomers in protein misfolding diseases. *J Biol Chem* 2004; 279:46363-6.
- Krammer C, Suhre MH, Krammer E, Diemer C, Hess S, Schatzl HM, et al. Prion protein/protein interactions: fusion with yeast Sup35p-NM modulates cytosolic PrP aggregation in mammalian cells. *FASEB J* 2008; 22:762-73.
- Kazantsev A, Preisinger E, Dranovsky A, Goldgaber D, Housman D. Insoluble detergent-resistant aggregates form between pathological and nonpathological lengths of polyglutamine in mammalian cells. *Proc Natl Acad Sci USA* 1999; 96:11404-9.
- Busch A, Engemann S, Lurz R, Okazawa H, Lehrach H, Wanker EE. Mutant huntingtin promotes the fibrillogenesis of wild-type huntingtin: a potential mechanism for loss of huntingtin function in Huntington's disease. *J Biol Chem* 2003; 278:41452-61.
- Lee HJ, Patel S, Lee SJ. Intravesicular localization and exocytosis of alpha-synuclein and its aggregates. *J Neurosci* 2005; 25:6016-24.
- Kanu N, Imokawa Y, Drechsel DN, Williamson RA, Birkett CR, Bostock CJ, Brookes JP. Transfer of scrapie prion infectivity by cell contact in culture. *Curr Biol* 2002; 12:523-30.
- Gousset K, Schiff E, Langevin C, Marijanovic Z, Caputo A, Browman DT, et al. Prions hijack tunneling nanotubes for intercellular spread. *Nat Cell Biol* 2009; 11:328-36.
- Vella LJ, Sharples RA, Lawson VA, Masters CL, Cappai R, Hill AF. Packaging of prions into exosomes is associated with a novel pathway of PrP processing. *J Pathol* 2007; 211:582-90.
- Fevrier B, Vilette D, Archer F, Loew D, Faigle W, Vidal M, et al. Cells release prions in association with exosomes. *Proc Natl Acad Sci USA* 2004; 101:9683-8.
- Vorberg I, Raines A, Story B, Priola SA. Susceptibility of common fibroblast cell lines to transmissible spongiform encephalopathy agents. *J Infect Dis* 2004; 189:431-9.

61. Schatzl HM, Laszlo L, Holtzman DM, Tatzelt J, Dearmond SJ, Weiner RI, et al. A hypothalamic neuronal cell line persistently infected with scrapie prions exhibits apoptosis. *J Virol* 1997; 71:8821-31.
62. Butler DA, Scott MR, Bockman JM, Borchelt DR, Taraboulos A, Hsiao KK, et al. Scrapie-infected murine neuroblastoma cells produce protease-resistant prion proteins. *J Virol* 1988; 62:1558-64.
63. Rujano MA, Bosveld F, Salomons FA, Dijk F, van Waarde MA, van der Want JJ, et al. Polarised asymmetric inheritance of accumulated protein damage in higher eukaryotes. *PLoS Biol* 2006; 4:417.
64. Krammer C, Kremmer E, Schatzl HM, Vorberg I. Dynamic interactions of Sup35p and PrP prion protein domains modulate aggregate nucleation and seeding. *Prion* 2008; 2:99-106.
65. Paushkin SV, Kushnirov VV, Smirnov VN, Ter Avanesyan MD. Propagation of the yeast prion-like [psi⁻] determinant is mediated by oligomerization of the SUP35-encoded polypeptide chain release factor. *EMBO J* 1996; 15:3127-34.
66. Derkatch IL, Bradley ME, Zhou P, Chernoff YO, Liebman SW. Genetic and environmental factors affecting the de novo appearance of the [PSI⁺] prion in *Saccharomyces cerevisiae*. *Genetics* 1997; 147:507-19.
67. Moriyama H, Edskes HK, Wickner RB. [URE3] prion propagation in *Saccharomyces cerevisiae*: requirement for chaperone Hsp104 and curing by overexpressed chaperone Ydj1p. *Mol Cell Biol* 2000; 20:8916-22.
68. Kryndushkin DS, Alexandrov IM, Ter Avanesyan MD, Kushnirov VV. Yeast [PSI⁺] prion aggregates are formed by small Sup35 polymers fragmented by Hsp104. *J Biol Chem* 2003; 278:49636-43.
69. Jones GW, Tuite MF. Chaperoning prions: the cellular machinery for propagating an infectious protein? *Bioessays* 2005; 27:823-32.
70. Rikhvanov EG, Romanova NV, Chernoff YO. Chaperone effects on prion and nonprion aggregates. *Prion* 2007; 1:217-22.
71. Osherovich LZ, Cox BS, Tuite MF, Weissman JS. Dissection and design of yeast prions. *PLoS Biol* 2004; 2:86.
72. Salnikova AB, Kryndushkin DS, Smirnov VN, Kushnirov VV, Ter Avanesyan MD. Nonsense suppression in yeast cells overproducing Sup35 (eRF3) is caused by its non-heritable amyloids. *J Biol Chem* 2005; 280:8808-12.
73. Alexandrov IM, Vishnevskaya AB, Ter Avanesyan MD, Kushnirov VV. Appearance and propagation of polyglutamine-based amyloids in yeast: tyrosine residues enable polymer fragmentation. *J Biol Chem* 2008; 283:15185-92.
74. Alberti S, Halfmann R, King O, Kapila A, Lindquist S. A systematic survey identifies prions and illuminates sequence features of prionogenic proteins. *Cell* 2009; 137:146-58.
75. Coustou V, Deleu C, Saupe S, Begueret J. The protein product of the het-s heterokaryon incompatibility gene of the fungus *Podospora anserina* behaves as a prion analog. *Proc Natl Acad Sci USA* 1997; 94:9773-8.
76. Patel BK, Gavin-Smyth J, Liebman SW. The yeast global transcriptional co-repressor protein Cyc8 can propagate as a prion. *Nat Cell Biol* 2009; 11:344-9.